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Antimycobacterial activity of linoleic acid and oleic acid obtained from the hexane extract of the seeds of *Mesua ferrea* L. and their *in silico* investigation

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Tuberculosis is responsible for about 8 million deaths worldwide annually. The emergence of multidrug-resistant and extensively drug-resistant strains urgently requires the development of new drugs against tuberculosis. Drug discovery from plants against tuberculosis is an exciting area for exploration. In the present study, the fatty acids- linoleic and oleic acids isolated and identified from the seeds of the plant *Mesua ferrea* L. exhibited antimycobacterial activity. The analysis was done using Gas Chromatography-Mass Spectrometry and supplementary information was obtained using fourier transform-infra red and ^{1}H and ^{13}C nuclear magnetic resonance. The minimum inhibitory concentration of the purified fraction containing both the compounds was found to be 78 µg/mL. *In silico* molecular docking studies against the target proteins GlfT2, Inh A and mtKasB of *Mycobacterium tuberculosis* revealed high scores for both the compounds. Cytotoxicity studies of the compounds revealed no toxicity and high antioxidant activity was observed.

Keywords: Antituberculosis, Cytotoxicity, Docking, Medicinal plants, *Mesua ferrea* L.

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Introduction

Tuberculosis, an infectious disease, caused by the microorganism *Mycobacterium tuberculosis* has been infecting human beings since ages[1]. It has been found to be the leading cause of death amongst all infectious diseases afflicting humans[2]. The World Health Organization in its recent update on tuberculosis reported that in 2013 alone, there were nearly 9 million new TB cases of which 1.1 million cases included Human immunodeficiency virus-infected people. In 2013, 1.5 million died of tuberculosis which includes 5,10,000 women and 3,60,000 number of Human immunodeficiency virus-infected individuals[3]. In view of the alarming global assessment of the disease in the context of the emerging threats from multidrug resistance and extensively drug-resistant strains of *M. tuberculosis* against the conventional first and second line drugs, there is an urgent need for discovering/developing new antimycobacterial agents. The World Health Organization declared tuberculosis as an emerging disease in the early nineties due to the emergence of drug-resistant strains and the increase in the incidence of co-infection in Human immunodeficiency virus-infected patients[4,5]. The frontline antituberculosis drugs that are effective in treating acute tuberculosis are found to be inactive in eliminating *M. tuberculosis* during the persistent stages of latent infection[6]. Thus therapeutics directly targeting the persistent bacilli are also urgently needed. A new infection, reactivation of latent tuberculosis and the emergence of drug-resistant strains of *M. tuberculosis* led to the increased demand for new chemotherapy regimens[7]. No new drug has been discovered since the advent of rifampicin in 1963[8].

Natural products or their derivatives form an important avenue for the search of new antitubercular agents[7]. Plants as a source of natural products provide an exciting field for the discovery of new drug leads and new chemical entities. Plants have been utilized as medicines for thousands of years in many parts of the world[9]. Plant-based drug discovery resulted mainly in the development of anti-infectious agents and continues to forward many new leads now in clinical trials[10].

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In the present investigation, the antimycobacterial activity of the natural products present in the hexane extract, obtained from the seeds of *Mesua ferrea* L. is being highlighted and the compounds identified. *M. ferrea* was selected for the study because of its wide use as a traditional medicinal plant. In Assam, the plant is widely used as an antiseptic, blood purifier and as a worm controller[11]. The antioxidant, cytotoxicity and *in silico* docking investigation have also been carried out to identify the potent targets.

Materials and Methods

Bacterial strain

The strain *Mycobacterium smegmatis* MC^2155 was used for the study. The strain was purchased from the Microbial Type Culture Collection and Gene Bank, The Institute of Microbial Technology, Chandigarh. The mycobacterial strain was grown in Middlebrook 7H9 broth (Himedia, India) medium supplemented with 0.2 % Glycerol and 10 % Oleic acid-Albumin-Dextrose-Catalase (OADC) media as prescribed in the manufacturer's instruction. The strain was stored for further use by preparing glycerol stocks and kept at -86 °C. Before each experiment, the strain was checked for any contamination using the acid-fast staining procedure**.**

Plant collection and identification

The seeds of the plant *M. ferrea* were collected from the Tezpur University campus during the month of January-February 2012. The seeds were washed and dried to remove any impurities present. The plant is well known and was identified by Mr Jintu Sharma, Taxonomist and Senior Technical Assistant and a voucher specimen (No TUMBBT/2012/005) was deposited in the repository of Department of MBBT, Tezpur University.

Extraction of crude extracts

Solvent extraction with hexane was carried out in the Soxhlet apparatus for the isolation of the crude extract from the seeds of *M. ferrea* by using a slightly modified method previously described[12]. The solvent was evaporated using a Rotavapor (Hahn Shin Scientific, South Korea) and the hexane extract obtained was dried over anhydrous sodium sulfate to remove moisture.

Purification of the hexane extract

The hexane extract of the seeds of *M. ferrea* was purified using a silica gel (60-120 mesh size) column. A solvent mixture of petroleum ether and ethyl acetate in three different ratios, 10:90, 30:70 and 50:50 was used as the eluent. The purified oil was dried over anhydrous sodium sulfate and kept in a desiccator vacuum until further use.

Preparation of fatty acid methyl esters

Fatty acid methyl esters were prepared from the hexane extract of the seed for identification of the compounds. To obtain the methyl esters 1 mL of the hexane extract was taken in a test tube and mixed with 2 mL of chloroform, 4 mL of methanol and 500 µL of concentrated sulfuric acid and heated at 100 °C for about 2 hours. The extract-solvent-acid mixture was cooled to room temperature and 3-4 mL of hexane was added to it and mixed. The hexane fraction was collected and analyzed.

GC-MS analyses of the fatty acid methyl esters

The gas chromatography-mass spectrometry analyses of the fatty acid methyl esters were carried out in a Varian-3800 GC/FID coupled to a SATURN-2200 Mass spectrophotometer using a CP-Sil 5 CB low bleed/MS (30 m x 0.25 mm x 0.25 µm) column as described previously[13] with certain modifications. The temperature of the oven was set using the following conditions: initial temperature set at 80 °C was held for 1 minute, then increased at a rate of 8 °C/ min and held for 15 minutes. Exactly 1 µL of sample was injected for the analysis. Data acquisition was done for the mass range between 50-550 mu and the scan speed was 1 scan/sec. Compound identification was done using both the NIST library and the SATURN library available with the instrument and also the retention time of the peaks.

Fourier transform infrared analysis of the essential oil

Fourier transform infrared analysis of the essential oil was carried out to investigate the functional groups present. The analysis was carried out in a Perkin Elmer Spectrum 100 Series IR spectrophotometer using potassium bromide discs. The spectra were taken in the absorbance mode and each sample was scanned 8 times with a scan speed of 4 cm/ sec starting from 400–4000/ cm.

Nuclear magnetic resonance of essential oil

1H NMR and 13C NMR were carried out using a 400 MHz JEOL FT-NMR. Tetramethylsilane was the internal standard. NMR grade $CDCl_3$ was used as the solvent, while a pulse width of 9.5 µsec and acquisition time of 2.83 sec was taken. A total of 8 scans were performed.

Antimycobacterial activity of *M. ferrea* seed oil

The antimycobacterial activity of the seed hexane extract of *M. ferrea* was determined by using 96 well plates following the method previously described[14]. A stock solution of the hexane extract was prepared at a concentration of 20 mg/mL. Various concentrations of the extract were prepared in aliquots in DMSO by serial dilution from the stock solution keeping the final concentration of dimethyl sulfoxide at 1 %. Exactly 100 µL of each was added to each well. To this, 100 µL of the bacterial inoculum relating to 0.5 Mc Farlands Standard was added. Isoniazid (0.02 mg/mL) was taken as the positive control while dimethyl sulfoxide (1 %) was the negative control. Plates were then incubated at 37 °C for 24 hours. After the incubation period, 40 µL of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was added and again incubated for about 30 minutes. A colour change to blue indicated the growth of bacteria while no change in colour indicated the antibacterial effect of the hexane extract.

Cytotoxicity of the essential oil fraction

The cytotoxicity of the purified essential oil fraction was assayed against RAW 264.7 macrophage cell lines. The cells were cultured in Dulbecco's Modified Eagle's Medium complete media supplemented with 10 % Fetal Bovine Serum and 100 µL of penstrep (Penicillin:Streptomycin) antibiotic reagent (Sigma). Cells were seeded into 96 well plates (NUNC, USA) and allowed to grow until confluency was attained. To the seeded cells, essential oil was added in the concentration range of 1, 5, 10, 20, and 40 µL. The plates were incubated at 37 °C for 12 hours. 30 µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide was added to the plates. After incubation, any change in colour was indicative of toxicity of the hexane extract.

Antioxidant activity of the essential oil

Antioxidant activity of the essential oil was determined by the DPPH ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay as previously reported[15]. DPPH free radicals were prepared in methanol at a concentration of 0.004 %. Different concentrations (0.062-1 mg/mL) of the essential oil fraction exhibiting antimycobacterial activity from *M. ferrea* seed was taken. The absorbance was read at 517 nm in a spectrophotometer (Thermo Fischer) after incubation for 30 minutes in dark at room temperature. Inhibition of DPPH free radical in percentage (I %) was calculated using the following formula.

$$I\ \% = (A\ blank - A\ sample / A\ blank) \times 100$$

where 'A blank' is the absorbance of the control reaction containing all the reagents except the extract. 'A sample' is the absorbance of the extract, *i.e*., the test compound. The concentration of the extract that provided 50 % inhibition was calculated from the graph plotting inhibition percentage against the extract concentration. All the tests were carried out in triplicates. Ascorbic acid was taken as the standard.

***In silico* investigation**

Molecular docking

Molecular Docking studies were carried out using Molegro Virtual Docker (MVD) 5.0[16]. The scoring functions of the ligands and the H-bonds formed with the amino acids were used in the prediction of the binding affinities, different binding modes and orientation of the compounds in the active site(s) of the target proteins. The water molecules were not taken into account in the study. The cavity detection algorithm in MVD was used for optimizing the potential binding site(s), *i.e*., it enables to define an approximate location of the most likely interaction sites. A set of 100 runs was given for each docking study using 2000 interactions. Both the Rerank scores and the MolDock scores were calculated. The best-fit score was obtained on the basis of the Moldock score[17].

Selection of the target proteins

The target proteins chosen for the study were downloaded from the Protein Data Base[18]. Three target proteins, Inh A (2B35), mtKas B (2GP6) and Glft2 (4FIX) were considered for the study based on their activity of synthesizing mycolic acids in both *M. tuberculosis* and *M. smegmatis.* Inh A is the enoyl ACP reductase protein of the mycobacterial type II fatty acid biosynthesis pathway. Inh A is the target of the first line anti-tuberculosis drug isoniazid. Inh A inhibition impairs the cell wall integrity which leads to the death of the cell and finally death of the organism[19]. The *M. tuberculosis* Kas B is a β ketoacyl acyl carrier protein synthase (ACP) II which is an elongation condensing enzyme present in the fatty acid synthase II (FAS-II) pathway. The Kas B along with Kas A is responsible for the fatty acid elongation in the FAS II pathway. Therefore the Kas B protein is considered as a potential target for anti-tuberculosis

drug candidate[20]. GlfT2 (Rv 3808c) is a polymerizing galactofuranosyltransferase which is involved in the synthesis of a variety of the galactan portion of the mycolyl arabinogalactan (MAG) complex in *M. tuberculosis.* This galactan is the attachment site of three different arabinan domains each of which contains ά- (1→ 5), ά- (1→ 3) and β - (1→ 2) linked arabinofuranose residue[21].

Results

Purification of the essential oil from *M. ferrea* seed

Column chromatography

The column chromatographic purification led to the identification of the active fraction-F2 which was further investigated to identify the compounds present within the fraction. The seed extract of *M. ferrea* in hexane was purified using different solvent ratios of petroleum ether and ethyl acetate. The maximum amount of the extract was eluted in the petroleum ether: ethyl acetate 10:90 solvent medium. The elution was divided into three fractions (F1-F3). Each of the fractions was again tested for their antimycobacterial activity using the MIC activity test. It was found that the fraction F2 possessed antimycobacterial activity while fraction F1 showed limited activity and F3 no activity. Therefore the fraction F2 was further analyzed using GC-MS to identify the compounds present.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS studies led to the identification of the active compounds. The F2 fraction obtained from column chromatography was converted into fatty acid methyl esters (FAMEs). Exactly 1 µL of the FAMEs was injected into the GC-MS and analyzed to investigate the presence of the fatty acid components. The fatty acid components: palmitic acid, linoleic acid, oleic acid, stearic acid and arachidic acid were found to be present in the F2 fraction, along with some other unidentified compounds (Fig. 1). From the results, it was inferred that the compounds linoleic acid and oleic acid (Fig. 2) were responsible for the antimycobacterial activity of the fraction. Both the essential fatty acids have been mentioned in the literature for their antimycobacterial activity[2]. The fraction F2 was further analyzed using FT-IR and NMR.

FT-IR of the F2 fraction

The FT-IR of the essential oil extracted from the seeds of *M. ferrea* was carried out to characterize the

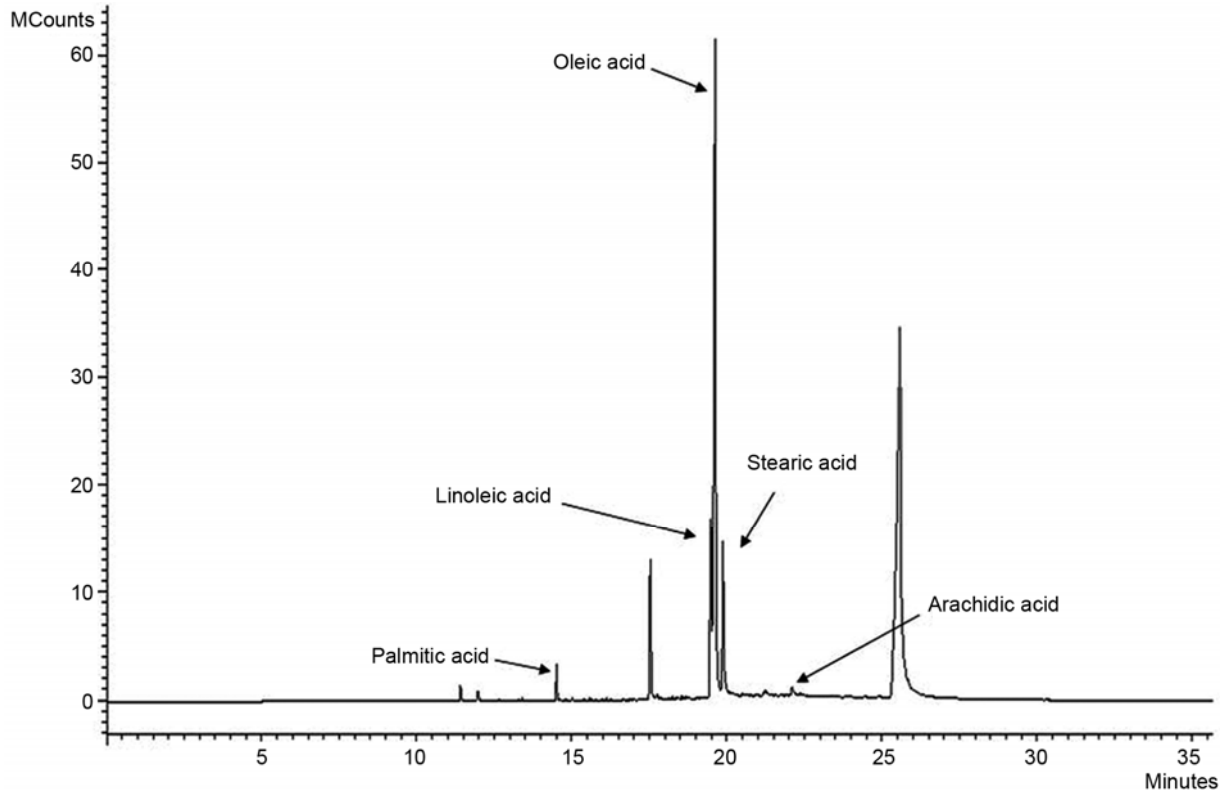

Fig. 1 — GC-MS of hexane extract from the seed of *Mesua ferrea* L.

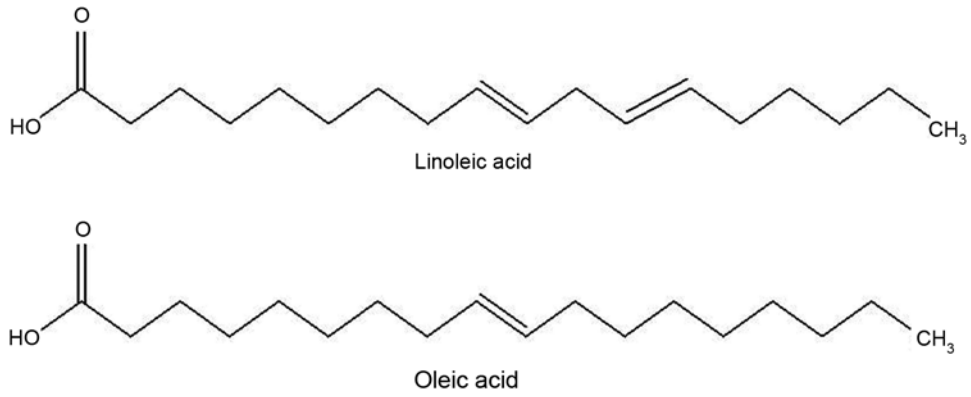

Fig. 2 — Structures of linoleic acid and oleic acid present in the essential oil fraction.

essential oils. The absorbance could be observed at 726, 1379, 1582, 1623, 1747, 1750, 3007, 3467/cm. The FT-IR studies indicated the presence of various functional groups associated with the unsaturated fatty acids. The transmittance at 1379, 1582, 1747, 1750/cm was due to the presence of symmetric and asymmetric C=O stretching vibration attributed to the carboxylic acids and at 726 and 1623/cm is due to the presence of alkene group. The presence of the hydroxyl groups could be observed at 3007 and 3467/cm.

NMR of the F2 fraction

The ^{1}H NMR and the ^{13}C NMR predicted the presence of different carbon and proton shifts indicative of the presence of both the linoleic and oleic acid.

^{1}H NMR and ^{13}C NMR

The presence of the signals from 5.31-5.9 ppm is due to the presence of oleifinic protons of isolated double bonds and conjugated double bonds[29]. The presence of the α-CH_2 protons and presence of methylene groups adjacent to the carbonyl group can be seen with the signals at 2.2-2.5 ppm while CH_2-CH=CH is observed with the presence of the signal at 2.0 ppm. The signals from 1.2-1.6 ppm and 0.83-0.98 ppm reflect the presence of $(CH_2)_n$ and CH_3 protons which are due to the presence of terminal methyl resonance (Fig. 3)[2,22]. ^{13}C NMR analysis revealed the presence of all the major groups present in the fatty acids. All the characteristic peaks as previously described have been found to be present in the analysis. In the analysis of fatty acids (lipids) the characteristic signals of ^{13}C NMR are grouped in four different regions. From 172-178 ppm the carbonyl and carboxyl carbons are present while from 124-134 ppm the unsaturated carbons are found, the glycerol backbone carbons can be observed in between 60–72 ppm and finally from 10-35 ppm the aliphatic carbons are found. In the present study, all the characteristic signals have been found to be present. From 14.19-32.00 ppm the presence of the aliphatic carbons, the 62.17-68.91 presence of glycerol backbone carbon, 129.76-130.09 demonstrated the presence of unsaturated carbons and the carbonyl and carboxyl carbons in between 172.98-173.43. The δ_C at 24.8, 25.7 and 27.3 is due to the presence of the saturated monoene and diene chain, a total number of diene chains and twice the total number of monoene and diene chains. The δ_C at 129.76 and 129.78 is due to the presence of the C_{10} and C_9 of the oleic chain while the smaller signals at δ_C 130.09, 129.9, 128.1 and 127.9 were due to the C_{13}, C_9, C_{10} and C_{12} of linoleic chain present in the essential oil component (Fig. 4)[2,29]. Thus the presence of both oleic acid and linoleic acid are found to coincide with the details documented in the literature which correlated with our analysis and demonstrated their presence in the hexane extract.

MIC of the purified compounds

The MIC of the essential oil fraction revealed the minimum concentration at which the growth of the bacteria was inhibited. It was found that the linoleic and oleic acid containing essential oil fraction of *M. ferrea* inhibited the bacteria at 78 µg/mL. The minimum inhibitory concentration (MIC) of the positive fraction containing both the compounds were found to match with the literature. The inhibitory mechanism of the fraction showing the antimycobacterial property is due to its detergent like properties[2].

Cytotoxicity assay of the purified compounds

The purified essential oil was investigated for their cytotoxicity using RAW 264.7 macrophage cell lines.

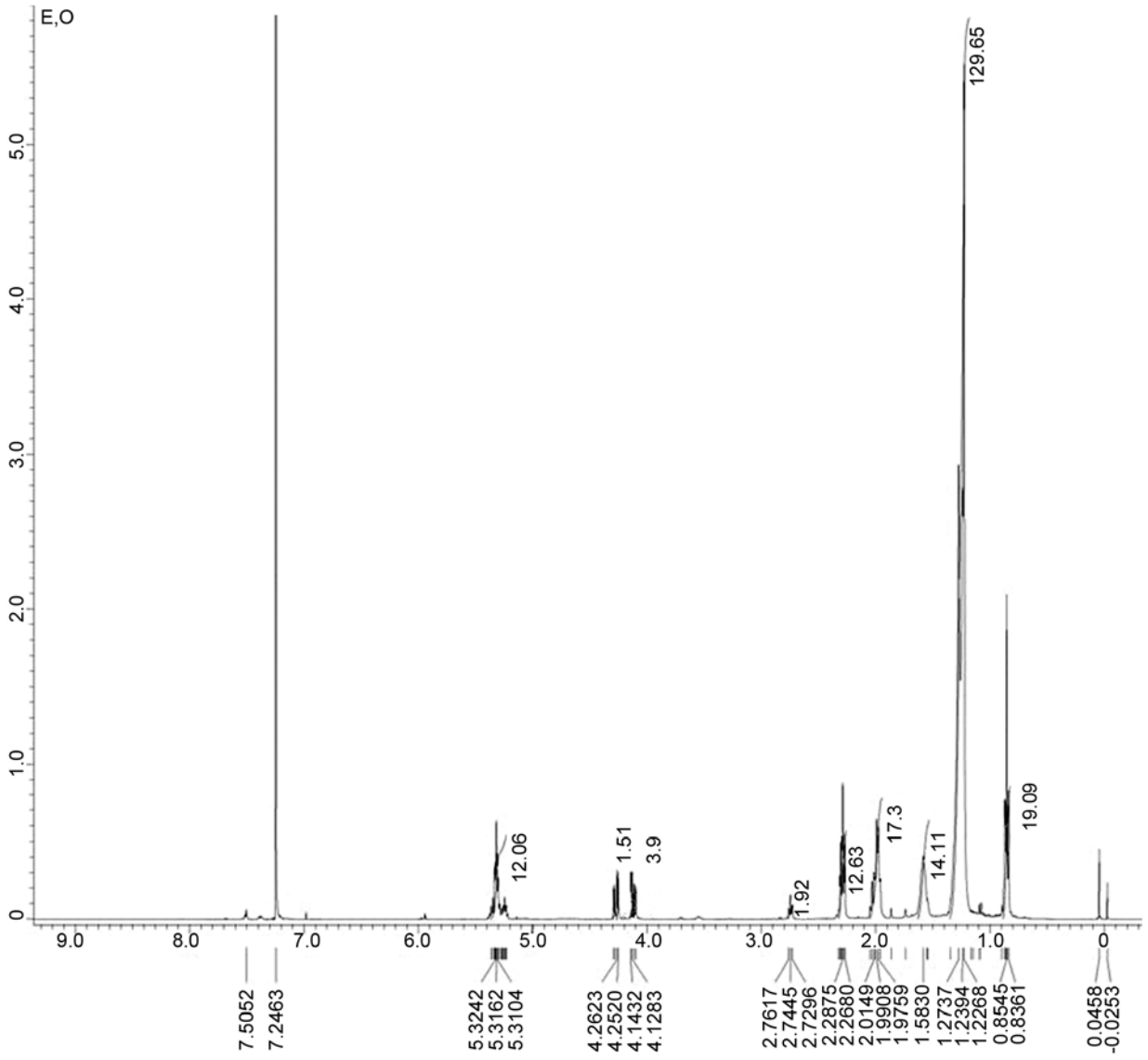

Fig. 3 — ^{1}H NMR spectra of the antimycobacterial purified fraction.

The essential oil was applied directly at different concentrations of 1,5,10, 20 and 40 µL into the 96 well plates in which the cells were seeded. The plates were incubated in a CO_2 incubator at 37 °C for 12 hours and then estimated for cytotoxicity using MTT. None of the concentrations of the essential oil was found to be toxic to the cells thus indicating that the compounds were not cytotoxic to the cell line Raw 264.7.

Antioxidant assay of the purified compounds

The antioxidant activity of the essential oil fraction containing linoleic acid and oleic acid were assayed using the DPPH free radical scavenging method. For the essential oil fraction, the concentrations ranged from 0.062-1 mg/mL. It was observed that at the highest concentration the oil fraction showed more than 93 % inhibition of DPPH free radical. Ascorbic acid taken as the positive control showed 97 % inhibition, slightly more than the tested samples. Thus the antioxidant studies of the compounds also indicated high radical scavenging activities of the compounds thus suggesting that the compounds are able to inhibit the formation of free radicals. The higher antioxidant property of the essential oil indicated pharmacological significance.

***In silico* investigation of the identified compounds**

Linoleic acid and oleic acid identified as the antimycobacterial compounds from the F2 fraction of *M. ferrea* L. were docked with the target proteins with 2000 iterations. The efficiency of docking is predicted

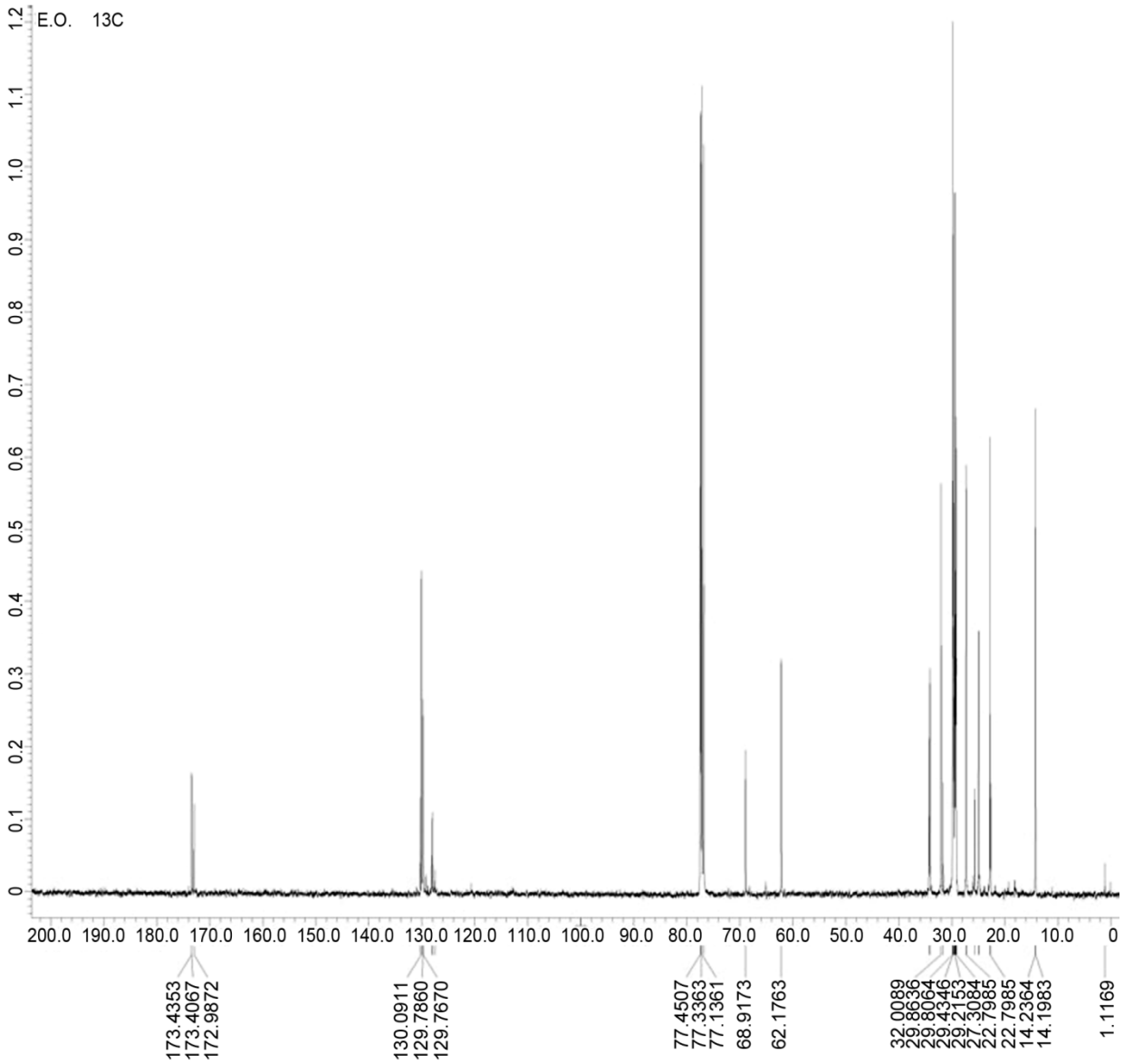

Fig. 4 — ^{13}C NMR spectra of the antimycobacterial purified fraction.

by the scoring functions from which the biological activity of the molecules can be evaluated[23].

Both linoleic and oleic acids exhibited different docking affinities (Fig. 5 and 6) with the galacto-furanosyltransferase protein from *M. tuberculosis.* Linoleic acid exhibited a MolDock score of -102.787 and a Rerank score of -85.5168 while oleic acid demonstrated a score of -101,424 and a Rerank score of -82.1008. The amino acid residues present in the cavity of the target protein which interacted with linoleic acids are Glu 300, Tyr 344 and Asp 372 with bond distances of 2.24 Å, 1.56 Å and 2.81 Å and those interacting with oleic acids are Lys 369, Trp 348 and Tyr 236 with bond distances of 1.77 Å, 2.59 Å and 2.74 Å. In case of Inh A, oleic acid demonstrated the best Moldock score of -112.575 and a Rerank score of -89.2977 while linoleic acid exhibited -103.844 and -83.5622 as Moldock and Rerank scores respectively. The residual amino acids interacting with the ligand linoleic acid were Arg 173, Ser 152, Tyr 259 and His 265 with bond distances of 2.52 Å, 2.61 Å 2.91 Å and 2.69 Å while the interacting amino acids with oleic acid were Asp 150, Ser152, Tyr 259, Ile 257, His 265, Asp 256 with bond distances of 3.62 Å, 1.87 Å, 3.07 Å, 1.98 Å, 2.84 Å and 2.12 Å. In the case of Inh A. commonality was observed with respect to the amino acid residues in both linoleic acid and oleic acid- Ser152, Tyr 259,

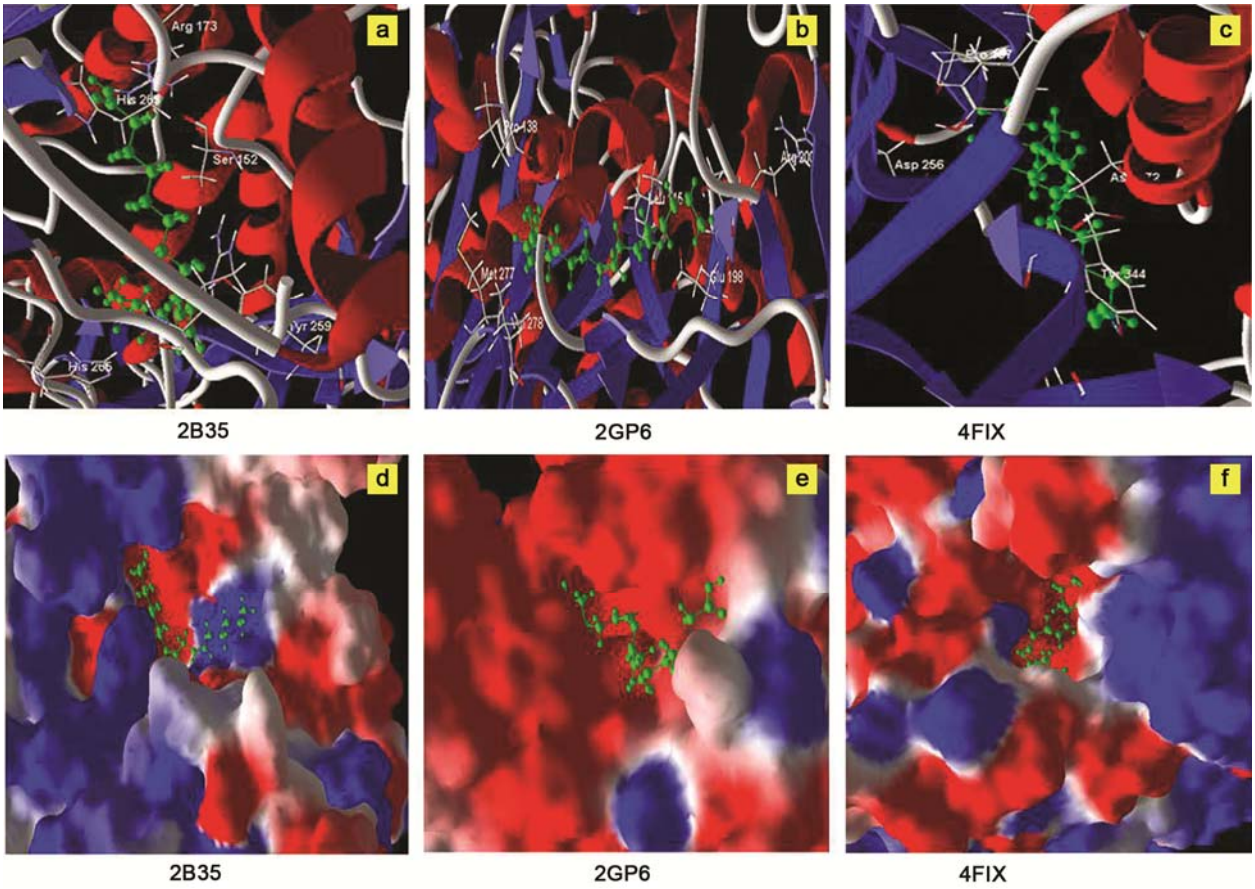

Fig. 5 — Docking study of linoleic acid with 2B35 (a,d), 2GP6 (b,e) and 4FIX (c,f) where a,b,c represents protein target in 2° structure view and d,e,f represents ligand docking within the protein cavity.

His 265 interacting with the ligands. In the case of mtKas B, Pro 138, Val 278, Met 277, Arg 200 and Glu 198 were found to the interacting amino acid residues having bond distances of 3.22 Å, 2.31 Å, 2.27 Å, 2.55 Å and 2.73 Å with linoleic acid which docked with the target protein with a Moldock score of -114.496 and a Rerank score of -59.0288 while Oleic acid docked with a Moldock score of -91.369 and a Rerank score of -24.2144 and Gly 406, His 408, Asn 147, Phe 405, Gln 142, Glu 198 and Gly 240 as the residual amino acids interacting with the molecule having bond distances of 2.21 Å, 3.24 Å, 3.07 Å, 1.89 Å, 2.98 Å, 2.97 Å and 3.43 Å.

The Moldock calculation is based on a score which is derived from PLP scoring function[24,27]. Interestingly the MolDock scoring function further improved the scoring functions with new charge schemes and hydrogen bonding terms. E_{score}, the docking scoring function is defined as

$$E_{score} = E_{inter} + E_{intra}$$

where, E_{inter} is the ligand-protein interaction energy and E_{intra} is the internal energy of the ligand.

The Rerank score obtained is actually a linear combination of E_{inter} (Van der Waals, electrostatic, hydrogen bonding, steric) between the protein and the ligand and E_{intra} (hydrogen bonding, torsion, Van der Waals, sp2-sp2, electrostatic) of the ligand weighted by predefined coefficients [26].

Therefore, the potential of the compounds as antimycobacterial was further justified on the basis of their *in silico* docking studies. The three target proteins chosen, play very important roles with reference to mycolic acid synthesis which is essential for the maintenance of the impermeability of the cell wall by most drugs. However, it was observed that both the compounds docked with all the three target proteins- Glf T2, Inh A and mtKas B. Linoleic acid docked better in case of Glf T2 and mtKas B while in the case of Inh A, oleic acid was found to be efficient than linoleic acid.

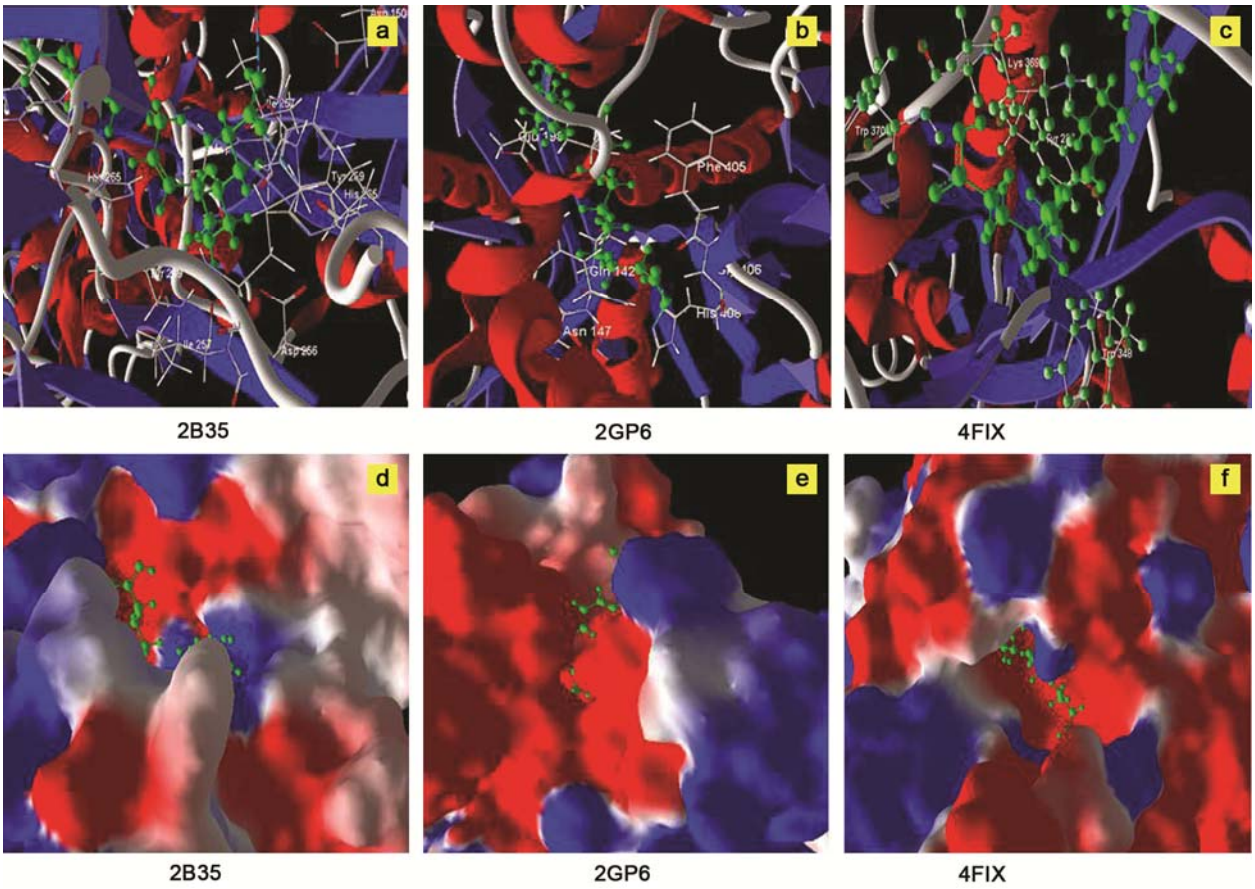

Fig. 6 — Docking study of oleic acid with 2B35 (a,d), 2GP6 (b,e) and 4FIX (c,f) where a,b,c represents protein target in 2° structure view and d, e, f represents ligand docking within the protein cavity.

Discussion

Medicinal plants have been widely investigated over the years for their potentiality in the treatment of several diseases including tuberculosis. Natural products obtained from plants have been the main source of alternative drugs for the treatment of several human ailments. Plants produce a variety of secondary product molecules like phenolics, flavonoids, terpenoids etc which have different pharmacological properties.

Linoleic acid and oleic acid identified from the hexane extract of the *M. ferrea* plant has been reported earlier to possess antimycobacterial properties[28]. The minimum inhibitory concentration of the fraction containing the active compounds correlated with earlier findings[28].

The presence of the compounds in the hexane extract has been demonstrated which is responsible for the antimycobacterial activity of the hexane extract. Gas chromatographic-mass spectrometric analysis revealed the presence of linoleic and oleic acid in the hexane extract. Fourier transform infrared analysis revealed the different functional groups, representative of both linoleic acid and oleic acid, present in the active fraction which yielded the compounds. Nuclear magnetic resonance (NMR) studies revealed the presence of different fatty acids in the hexane extract including oleic acid and linoleic acid. NMR is a widely used instrument in the analysis of fatty acids. Both ^{1}H NMR and ^{13}C NMR are used in the identification of molecules without going for individual isolation. Different signals were visualized from both the ^{1}H NMR and ^{13}C NMR study which revealed the presence of both linoleic and oleic acid in the hexane extract. *In silico* docking studies revealed the potential docking of the active compounds in the target proteins which demonstrated their antimycobacterial properties. Efficient docking of linoleic and oleic acid with the target proteins involved in mycolic acid synthesis reveals the potential activity of both the molecules.

The active fractions were found to be non-cytotoxic suggesting their potential advantage over other synthetic drugs and also demonstrated high antioxidant activity.

Thus the present study demonstrated the presence of the compounds linoleic acid and oleic acid in the hexane extract of the *M. ferrea* seeds which were responsible for the anti-mycobacterial against *M. smegmatis.*

Conclusion

Plants are a rich storehouse of diverse chemical compounds. Drug-like molecules obtained from plants offers an alternate choice over conventional synthetic drugs. In the present study, the search for antimycobacterial plant metabolites led to the identification of linoleic and oleic acid from *M. ferrea* L. Characterization of the compounds was carried out using GC-MS, FT-IR and ^{1}H and ^{13}C NMR studies. No toxicity was observed against the Raw 264.7 cell line. Docking studies using the *M. tuberculosis* target proteins GlfT2 and Inh A indicated the docking efficacy of linoleic acid over oleic acid with better docking scores of -54.0174 and -79.1535 as against -33.5739 and -74.884 exhibited by oleic acid. The MIC study of the purified fraction containing both the compounds were found to be higher with a concentration of 78 µg/mL which suggested that the compounds can be further studied for forwarding them as potential anti-mycobacterial agents. The antioxidant study also revealed the high antioxidant potential of the compounds which might be of pharmacological advantage.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest between them.

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